

What we claim is:

1: A method for quantifying the amount of at least one target protein in a sample comprising the steps of:

- 5           1. digesting the sample to provide peptides,
2. preparing at least one labeled monitor peptide comprising a subsequence of said target protein(s) to provide an internal standard,
3. adding an aliquot of the product of step 2 containing a known amount of labeled synthetic peptide to the product of step 1,
- 10          4. loading the product of step 3 onto at least one support which has attached thereto at least one binding agent which binds to at least one monitor peptide,
5. washing the support(s) of step 4 to remove unbound peptides,
6. eluting the monitor peptide (s) from the binding agent(s) bound to the support (s),
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2: The method of claim 1 wherein the label is a stable isotope or mixture thereof

3: The method of claim 2 wherein the isotope is  $^{15}\text{N}$ .

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4: The method of claim 2 wherein the isotope is  $^{13}\text{C}$ .

5: The method of claim 2 wherein the isotope is  $^{18}\text{O}$ .

6: The method of claim 1 wherein, in step 4, the binding agent is an antibody.

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7: The method of claim 6 wherein the antibody is a monoclonal antibody.

8: The method of claim 6 wherein the antibody is a polyclonal antibody.

9: The method of claim 1 wherein, in step 4, the binding agent is an RNA aptamer.

5 10: The method of claim 1 wherein the binding agent is a recyclable binding agent.

11: The method of claim 1 wherein the solid support is comprised of beads.

10 12: The method of claim 1 wherein the solid support is a packed column.

13: The method of claim 1 wherein the solid support is a monolithic porous support.

15 14: The method of claim 1 wherein the support has at least one openings said opening(s) having a surface to which the binding agents bind.

15: The method of claim 1 wherein the support to which the binding agents bind has a hydrophilic plastic.

20 16: The method of claim 1 wherein the support is a mesh.

17: The method of claim 1 wherein the support is a gel.

25 18: The method of claim 1 wherein the support to which the binding agent binds has a hydrophobic plastic surface.

19: The method of claim 1 wherein the flow path is a reconfigurable flow path.

30 20: The method of claim 1 wherein the flow path can be regulated between loading in step 4 and eluting in step 6 to allow each support to be eluted individually.

21: The method of claim 1 wherein, during step 7, the peptides are fragmented after introduction into the mass spectrometer.

22: The method of claim 1 wherein, in step 7, the peptides are not fragmented, but the peptides are detected in the form in which they were introduced into the mass spectrometer.

5 23: The method of claim 1 wherein different labeling atoms are used to label a specific synthetic monitor peptide so that the labeled monitor peptides having the same amino acid sequence differ in mass.

10 24: The method of claim 1 wherein at least two different labeled monitor peptides are prepared in step 2, the mixture of labeled monitor peptides being added to the product of step 1, and thereafter, in step 4, the product of step 3 is loaded on a support system with multiple species of binding agents, at least one such binding agent binding preferentially to a particular monitor peptide.

15 25: The method of claim 24 wherein the differing monitor peptides are selected for optimal differential detection in the mass spectrometer.

20 26: The method of claim 1 wherein at least two different labeled monitor peptides are prepared in step 2, the mixture of labeled monitor peptides being added to the product of step 1, and thereafter, in step 4, the product of step 3 is loaded sequentially onto support, each having binding agents which preferentially bind to one particular sequence, each support being, thereafter, subjected to elution and each eluate sample thereafter being introduced separately into the mass spectrometer.

25 27: A method for capturing similar amounts of 2 or more different monitor peptides from a sample containing widely varying concentrations of the different monitor peptides comprising the steps of:

- 30 1: fragmenting the proteins of the samples,  
2: producing labeled monitor peptides,  
3: adding the product of step 1 to the product of step 2 containing a known amount of labeled monitor peptide,  
4: preparing at least one support having at least one binding agent which binds a small fraction of the more abundant peptide in the sample and at least one binding agent which binds higher fraction of the less abundant peptide in the sample,

- 5: loading the product of step 3 onto the support prepared in step 4,
- 6: washing the support of step 5, then
- 7: eluting the peptides from the support to obtain the monitor peptides, and
- 8: subjecting the eluate of step 7 to mass spectrometry.

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28: A support system for collecting more than one monitor peptide from a peptide sample wherein at least one monitor peptide is present in the sample in much greater concentration than at least one other monitor peptide, said support having attached thereto a first binding agent being applied to said support to bind only a small fraction of the monitor peptide of higher concentration and a second binding agent being applied to said support to bind a higher fraction of the monitor peptide of low concentration in the sample.

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29: The support of claim 28 wherein the proportional amount of each monitor peptide bound is dependant on the amount of each binding agent.

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30: The support of claim 28 wherein the proportional amount of each monitor peptide bound is dependant on the affinity of each binding agent.

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31: A kit comprising at least one labeled monitor peptide wherein the kit further comprises at least one binding agent for capture of at least one monitor peptide.

32: A method of measuring the progress of proteolytic digestion of a sample comprising the steps of:

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1: selecting at least two monitor peptides derived from the sequence of one known protein in the sample, wherein at least one of said monitor peptides is known to be released earlier in the time course of a reference digest relative to at least one other monitor peptide,

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2: measuring the amount of said monitor peptides at one or more time points during digestion, and

3: comparing the relative amounts of said monitor peptides in a mathematical process to compute an index reflecting the degree to which digestion has progressed relative to the time course of said reference digest.

33: The method of claim 32 further comprising the use of said index to determine the acceptability of a digest of a sample for the purpose of inferring the amount of at least one protein in said sample based on measurements of at least one monitor peptide derived from the sequence of said protein.

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34: The method of claim 32 further comprising the use of said index to correct the measurement of at least one monitor peptide in said digest to estimate the measurement which would have been obtained if the digest had proceeded to the same extent as the reference digest at a specified point in said reference digest timecourse.

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35: A method of quantifying the amount of at least one target protein in a sample comprising the steps of:

1: digesting the sample to provide peptides,

2: preparing at least one labeled synthetic monitor peptide comprising a sequence of said target protein(s) to provide an internal standard,

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3: adding an aliquot of the product of step 2 containing a known amount of labeled monitor peptide to the product of step,

4: loading the product of step 3 onto at least one support which has attached thereto at least one binding agent which binds to at least one monitor peptide,

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5: washing the support(s) of step 4 to remove unbound peptides,

6: eluting the monitor peptide(s) from the binding agent(s) bound to the support(s) of step 4,

7: loading the monitor peptides eluted in step 6 onto a second support which has attached thereto at least one binding agent different from the binding agent(s) of step 4 and which binds to at least one monitor peptide,

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8: washing the support(s) of step 7 to remove unbound peptides,

9: eluting the monitor peptides from the binding agent(s) bound to the support(s) in step 7, and

10: subjecting the eluate obtained in step 9 to mass spectrometry to determine the amount of monitor peptide(s) in the eluate in comparison to the labeled synthetic standard.

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36: A method of characterizing the structure of a target protein in a sample, said method comprising the steps of:

- 1: subjecting an aliquot of said sample to a denaturing process,
  - 2: digesting the product of step 1,
  - 3: digesting a second aliquot of said sample without denaturing,
  - 4: preparing at least one labeled synthetic monitor peptide comprising a  
5 sequence of the target protein to provide an internal standard,
  - 5: to the product of step 2 add a known amount of the product of step 4,
  - 6: to the product of step 3 add the same known amount of the product of step  
4,
  - 7: load each of the products of steps 5 and 6 onto separate supports which  
10 have attached thereto at least one binding agent which binds to the monitor peptide,
  - 8: wash each support prepared in step 7,
  - 9: elute the peptides from each support,
  - 10: subject each eluate obtained in step 9 to mass spectrometry, and
  11. compare the amount of monitor peptide in each of the eluates.
- 15 37: The method of claim 1 wherein, in step 2, the monitor peptide is a synthetic peptide.
- 20 38: The method of claim 1 wherein the label the label is introduced by chemical modification of a natural peptide.
- 39: The method of claim 38 wherein the chemical modification results in the attachment of additional atoms to the monitor peptide.
- 25 40: The method of claim 39 wherein the binding agent is selected to bind to the chemically modified structure of the monitor peptide.
- 41: The method of claim 40 wherein sample peptides are subjected to same  
30 chemical modification but without the introduction of the label
- 42: The kit of claim 31 containing, additionally, a support.
- 43: The kit of claim 42 wherein the binding agent is attached to the support.